TGF-B-RECEPTOR ECTODOMAIN FUSION MOLECULES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and uses thereof. More specifically, the present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and their use in TGF-β superfamily ligand neutralization.

BACKGROUND OF THE INVENTION

[0002] TGF-β is part of a superfamily of over 30 ligands that regulate several physiological processes, including cell proliferation, migration and differentiation. Perturbation of their levels and/or signaling gives rise to significant pathological effects. For instance, TGF-β and activin ligands play critical pathogenic roles in many diseases including cancer (Hawinkels & Ten Dijke, 2011; Massague et al, 2000; Rodgarkia-Dara et al, 2006). TGF-β, in particular, is considered as a critical regulator of tumor progression and is overexpressed by most tumor types. It favors tumorigenesis in part by inducing an epithelial-mesenchymal transition (EMT) in the epithelial tumor cells, leading to aggressive metastasis (Thiery et al, 2009). TGF-β also promotes tumorigenesis by acting as a powerful suppressor of the immune response in the tumor microenvironment (Li et al, 2006). In fact, TGF-β is recognized as one of the most potent immunosuppressive factors present in the tumor microenvironment. TGF-β interferes with the differentiation, proliferation and survival of many immune cell types, including dendritic cells, macrophages, NK cells, neutrophils, B-cells and T-cells; thus, it modulates both innate and adaptive immunity (Santarpia et al, 2015; Yang et al, 2010). The importance of TGF-beta in the tumor microenvironment is highlighted by evidence showing that, in several tumor types (including melanoma, lung, pancreatic, colorectal, hepatic and breast), elevated levels of TGF-β ligand are correlated with disease progression and recurrence, metastasis, and mortality. Hence, significant effort has been invested in devising anti-tumor therapeutic approaches that involve TGF-β inhibition (Arteaga, 2006; Mourskaia et al, 2007; Wojtowicz-Praga, 2003).

[0003] One approach to developing therapeutic agents that inhibit TGF-β function has been to use antibodies or soluble decoy receptors (also termed receptor ectodomain (ED)-based ligand traps) to bind and sequester ligand, thereby blocking access of ligand to its normal cell surface receptors (Zwaagstra et al, 2012). In general, receptor ED-based traps are a class of therapeutic agents that are able to sequester a wide range of ligands and that can be optimized using protein engineering approaches (Economides et al, 2003; Holash et al, 2002; Jin et al, 2009).

[0004] Previously, a novel protein engineering design strategy was used to generate single-chain, bivalent traps that are able to potently neutralize members of the TGF-β superfamily of ligands due to avidity effects (Zwaagstra et al, 2012) [WO 2008/113185; WO 2010/031168]. In this case, bivalency was achieved via covalent linkage of two TβRII ectodomains using portions of the intrinsically disordered regions (IDR) that flank the structured, ligand-binding domain of TβRII-ED. One example of these single-chain bivalent traps, T22d35, exhibited TGF-β neutralization potencies ~100-fold higher than the monovalent non-engineered TβRII ectodomain, though it did not neutralize the TGF-β2 isoform and had a relatively short circulating half-life.

[0005] While research to date indicates that single-chain TGF-β traps have promising therapeutic potential, their circulating half-lives and manufacturability present challenges to the commercial application.

SUMMARY OF THE INVENTION

[0006] The present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and uses thereof. More specifically, the present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and their use in TGF-β superfamily ligand neutralization.

[0007] In some aspects, the invention relates to TGF-β superfamily receptor ectodomain-based polypeptides that are similar to typical Fc fusions in design, in that the ectodomain is fused to a dimeric antibody constant domain. In particular, with respect to the present polypeptides, the Fc portion occupies the N-terminal position. Fc fusions in the prior art typically provide the Fc portion at the C-terminal end of the fusion. As will be evident from the results presented herein, this difference in orientation provides a number of significant advantages.

[0008] In other aspects, the present polypeptides incorporate at least two TGF-β superfamily receptor ectodomains that are linked in tandem to the C-terminus of an antibody constant domain.

[0009] Thus, there is provided a polypeptide construct comprising: a first portion comprising the second constant domain (C_{H2}) and/or third constant domain (C_{H3}) of an antibody heavy chain, and a second portion comprising at least two TGF-β superfamily receptor ectodomains (TβSR-ED) linked in tandem; wherein the N-terminus of the second portion is linked to the C-terminus of the first portion.

[0010] There is also provided a polypeptide construct comprising: a first portion comprising the second constant domain (CH2) and/or third constant domain (CH3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomains (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and further wherein the first portion does not further comprise an antibody that binds to an antigen that is PD-L1, EGFR1, Her-2, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, or CTLA-4.

[0011] There is provided a polypeptide construct comprising: a first portion comprising the second constant domain (C_{H2}) and/or third constant domain (C_{H3}) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED), wherein the N-terminus of the second portion is directly fused to the C-terminus of the first portion.

[0012] In an embodiment, there is provided a polypeptide construct comprising a first portion comprising the second constant domain (C_{H2}) and/or third constant domain (C_{H3}) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and wherein the polypeptide construct neutralizes TGF-β with at least 100-fold more potency than the TβSR-ED alone.

[0013] In a preferred embodiment, the second portion comprises one, two, or multiple TGF-β superfamily receptor ectodomain (TβSR-ED). In a preferred embodiment, the